THE UNIVERSITY OF ALBERTA

THE EFFECT OF SUBLETHAL DOSES OF METHYLMERCURY

ON THE COLOR VISION OF RAINBOW TROUT

(*SALMO GAIRDNERI* RICHARDSON)

by

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Dedication

To those Native People in Northwestern Ontario
who have suffered.

ABSTRACT

Three toxicity experiments were conducted using single intra-peritoneal injections of methylmercuric chloride (MMC). The tissue content of methylmercury was measured by a total mercury analysis (μg Hg/g of tissue). The 15 day LD50 of MMC administered to rainbow trout by intraperitoneal injection was 7.1 ± 0.4 mg MMC/kg of body weight. Approximately 90 percent of the dose administered was retained for 15 days. Two experiments were conducted to examine the concentration of Hg in the brain, epaxial muscle, and whole eye; 30 days subsequent to a sublethal injection of methylmercuric chloride. The tissues differed in the magnitude of Hg uptake: brain > muscle > eye. The muscle concentration of Hg was similar to whole body concentrations of Hg ($p > 0.05$).

The psychophysical photopic spectral sensitivity was estimated for 15 rainbow trout using a two-choice operant conditioning technique (Phase I). Ten test wavelengths were utilized and ranged from 425 to 650 nm. The mean and normalized mean sensitivity curves appeared to have three components: one which ranged from 425 to 500 nm, a second from 525 to 575 nm, and a third from 600 to 650 nm. The peak sensi-tivities of these presumed receptive types are 475, 550, and 625 nm, respectively. Response latency did not vary with wavelength to any significant extent.

The subjects which were used in Phase I were then divided into four groups: control (saline) (n=4), the 0.3 toxic unit group (n=2), the 0.6 toxic unit group (n=4), and the 0.8 toxic unit group (n=5).

These individuals were given weight-specific injections of a particular dose of MMC or saline and remained inactive for a period of 15 days during which methylmercury accumulated in body tissues. A retest phase (Phase III) indicated that methylmercury did impair photopic spectral sensitivity of the test fish, notably at 475, 500, 525 and 600 nm. Due to the nature of this impairment it appears that methylmercury is exerting influence on the neural processes central to the photoreceptors.

ACKNOWLEDGMENTS

A very special thanks to Ma, Pa, and Kim Hawryshyn and all the other kin who always encouraged my intellectual development.

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Chapter I

GENERAL INTRODUCTION

Inorganic mercury in the aquatic environment, whether natural or introduced by man, tends to settle to the benthic sediments because it has a high molecular weight and low solubility. In the sediments, bacterial methylation of the inorganic mercury occurs (Wood et al., 1968). This methylated form of organic mercury enters the food chain (see Fig. 1) where it is biologically magnified, that is, a quantity of mercury is distributed among proportionally fewer animals as it proceeds through the food chain (Jernelov, 1969).

Methylmercury can easily cross biological membranes because it has a single valence, a relatively small size, a high solubility in lipid, and ample availability of sulfhydryl containing transport proteins in the blood (Hughes, 1957). Since mercury has a particularly high affinity for sulfhydryls, these transport proteins and various enzymes may become inactive in the presence of mercury. Approximately 85 to 95 percent of the total mercury in contaminated fish is in the form of methlymercury (Noren et al., 1967; Westöö, 1968). Fish can accumulate methylmercury through transcutaneous absorption, across respiratory surfaces and internally across digestive tissues (Hannerez, 1969). Since there is virtually no free methylmercury in solution of most water bodies, the consumption of methylmercury laden prey items is most likely the source of methylmercury accumulation.

Neuropathological damage appears to be the mode of toxicity of methylmercury (Berlin et al., 1973; Evans et al., 1977). This may be

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Figure 1. Distribution of mercury through the environment to its

incorporation into the food chain. (After Munson, 1978).

Concentration of mercury per animal increases

Fish eating birds

Fish

Higher invertebrates

Plankton

sediment feeders - Benthic organisms

Fish eating mammals

Carnivores

Herbivores

Hg compounds in solution

Plants

anaerobic bacterial transformation (methylation)

Sediment - Hg^{++} - insoluble salts

Terrestrial

Water

Sediment

Industrial Hg input

Industrial Hg input

a function of the high content of sulfhydryl groups in the nervous system and the irreversibility of mercury sulfhydryl complexes (Manalis and Cooper, 1975). Recent reports have demonstrated a disparity in the distribution of methylmercury; the occipital cortex lateral geniculate nucleus and corpus striatum possessed the highest concentration along with resultant lesioning in monkeys (Evans et al., 1977). This rationalizes the prevalence of ataxia and visual aberrations as symptoms of methylmercury poisoning (Suzuki et al., 1973).

Previous researchers have used rudimentary psychophysical visual measurements to assess the effect of methylmercury on vision. However, their results have not indicated what processes of vision were affected. Berlin et al. (1973) state that methylmercury tended to affect form discrimination (in primates) more than color discrimination but since they used high doses (20-50 mg Hg/kg body weight) and their measurements were simplistic, the results are not convincing. Lyle (1976) states that most neuropathological conditions in humans affect the normal process of color vision and that color opponent pairs of receptor types are usually affected. Thus, one might expect some detriment to the chromatic visual system as a consequence of methyl-mercury exposure.

Color vision requires the presence of more than one type of photoreceptive mechanism with different peak sensitivities (Daw, 1973). Photopic spectral sensitivity has been demonstrated by behavioral experimentation in goldfish (Yager, 1968; McCleary et al., 1969), sunfish (Hurst, 1953), carp (Oyama and Jitsumori, 1973) and rudd (Muntz and Northmore, 1970).

Color vision can augment visual acuity in certain environmental situations (Munz and MacFarland, 1975). Tropical fishes have visual pigments which are adapted to the spectral composition of underwater illumination so that there is a maximum absorption of the light available (Munz and MacFarland, 1975). Therefore a multiple receptive system promotes a greater sensitivity of contrasts to detect the movement or presence of objects against the background of light in an aquatic environment (Lythgoe, 1974). Since color vision probably plays a vital role in life sustaining processes such as mate recognition, spacing in schools, territoriality and selective predation, impairment of the photopic visual system could be detrimental to an organism.

My research objectives were to determine the toxicity and tissue uptake of methylmercuric chloride injected intraperitoneally in rainbow trout (*Salmo gairdneri* Richardson); to estimate the psychophysical photopic spectral sensitivity; and to examine the effects of sublethal doses of methylmercuric chloride on the photopic spectral sensitivity of rainbow trout.

Rainbow trout were utilized because they are classified as the standard test species in toxicological research. Since color vision of rainbow trout was virtually unexplored, I had to characterize the photopic visual system in order to examine the effects of methylmercury on its normal operation.

Chapter II

PSYCHOPHYSICAL PHOTOPIC SPECTRAL SENSITIVITY IN

RAINBOW TROUT (*SALMO GAIRDNERI*)

Introduction

Spectral sensitivity may be considered as a possible method of
diagnosing the effects of environmental contamination since it provides
a direct reflection of the state of the central nervous system (Lyle,
1976). As well, the photopic spectral sensitivity plays a vital role
in the behavior of an organism such as the perception of visual stimuli
occurring in life-sustaining activities (McFarland and Munz, 1975).
Rainbow trout was a good candidate for examination because of its
important role in aquatic toxicological research and also to provide
information about its color vision.

Psychophysical determinations of photopic spectral sensitivity
have been made in goldfish (Yager, 1967, 1969; Thorpe, 1971; Beauchamp
and Rowe, 1977) and the rudd (Muntz and Northmore, 1970, 1971;
Northmore and Muntz, 1974), indicating that these fish possess
trichromatic color vision. Ali (1974) suggests that brook trout
(*Salvelinus fontinalis*) may possess three anatomical cone types with
blue, green, and red sensitivities.

The objective of the present study was to provide preliminary
information concerning the photopic spectral sensitivity of rainbow
trout. The methodology of this experiment was based on a modification
of that employed by Yager (1967). In a two-choice situation, the trout
evidenced detection of a spectral stimulus by swimming into the lighted

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chamber and avoiding a dark chamber. These findings may facilitate future research in behavioral effects of toxicity with rainbow trout.

Materials and Methods

Subjects

The test animals, 20 rainbow trout, were obtained from the Sam Livingston Fish Hatchery at Calgary, Alberta, and measured 15 - 20 cm in length. The trout were held in 200 ℓ continuous flow-through tanks at a temperature of $15 \pm 0.5°C$ and a 10 hour light, 14 hour dark photoperiod (white fluorescent lamp F20) for at least two weeks prior to experimentation. The fish were housed in individual compartments to prevent stressful interaction with conspecifics.

Apparatus

The test tank was a glass aquarium 31 x 93 x 20 cm high divided into three areas of equal size, two response chambers and a neutral chamber (Fig. 2). The response chambers were delimited by transversely arranged barriers (black plexiglas 1/4") located 31 cm centrally from either end of the tank. Each barrier had a rectangular hole which allowed passage to either response chamber when the pulley-operated gates were opened. Stainless steel shocking grids present in both response chambers were vertically separated by 7 cm, insuring free mobility of the subject. The grids were wired to a SD-9 Grass electric stimulator which generated 2 square wave pulses at 5 - 15 mA; each pulse had a 50 msec duration and was separated by 800 msec.

Ten monochromatic stimuli from 425 - 650 nm with a 20 nm half intensity band width were projected onto circular stimulus patches at

Figure 2.   Schematic representation of apparatus.

bbs - broadband stimulus source; ce - chamber entrance;

f_1 - f_2 - 1 log and 2 log neutral density filters;

g - gates; m - monochromator; nc - neutral chamber;

rc - response chamber; sp - stimulus patch; w - neutral

density balancing wedge.
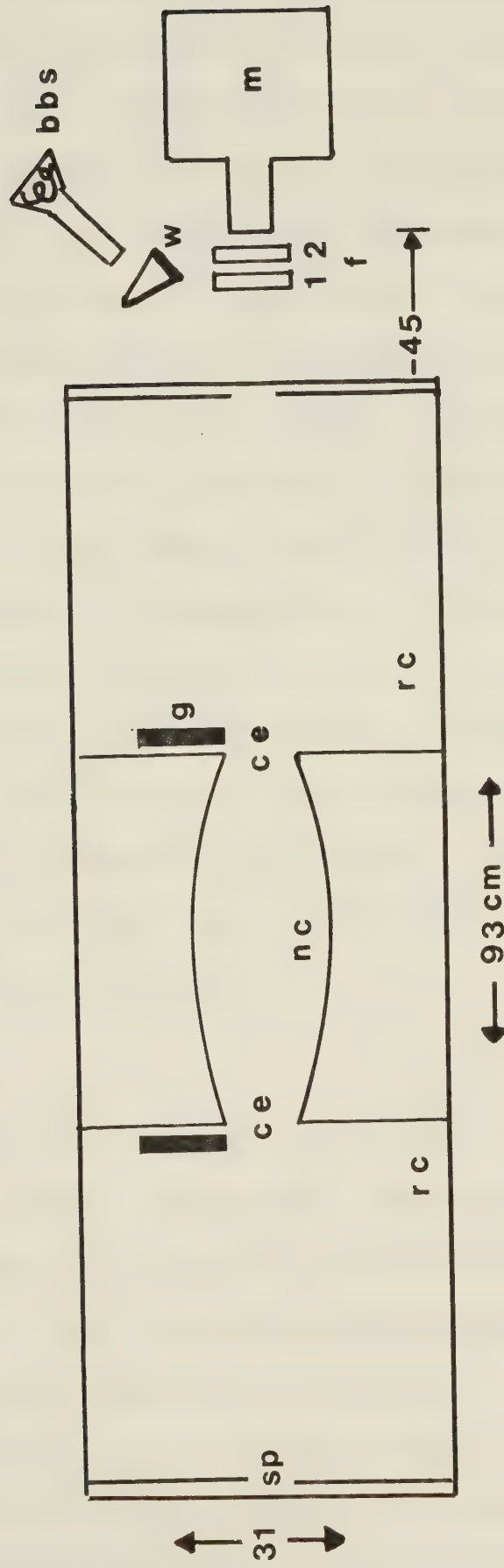
the end of each response chamber. These patches were constructed from plastic projection screen material and had a diameter of 4.7 cm subtending a visual angle of 8 degrees from the response chamber gates. Spectral radiant exitance (intensity) of the stimuli was adjusted by variable rheostats and calibrated with an ISCO model SR spectroradiometer inside the dry test tank using a remote optic probe.

Broad band light sources, used for purposes of spectrally neutral training, consisted of Vickers Instruments microscope lights with a spectral energy distribution illustrated in Figure 3. The intensity was controlled by neutral density wedges and filters to produce luminances of 1.20 and 0.52 (measured by an intensity meter and converted to luminance) nit during training. An incandescent lamp, situated over the neutral chamber was used as an adapting light to maintain photopic vision; its illuminance at the bottom centre of the neutral chamber was 3.77 lux (measured by an intensity meter). The spectral energy distribution of this light is also illustrated in Figure 3 (measured by a spectroradiometer).

Procedure

The operant conditioning paradigm that was employed is illustrated in Table I. The negative reinforcement (shock) was administered for all incorrect responses, whereas the positive reinforcement (small uniform pieces of beef heart) was administered approximately every fifth trial for correct responses (partial schedule).

Three groups of 6, 6, and 8 subjects were tested separately between February and July of 1978 of which five, four, and five, respectively, completed all the wavelength tests. Three fish were dropped because

Figure 3. Spectral energy distribution of the overhead adapting

lamp (o-o) and the broad band light source (●-●).
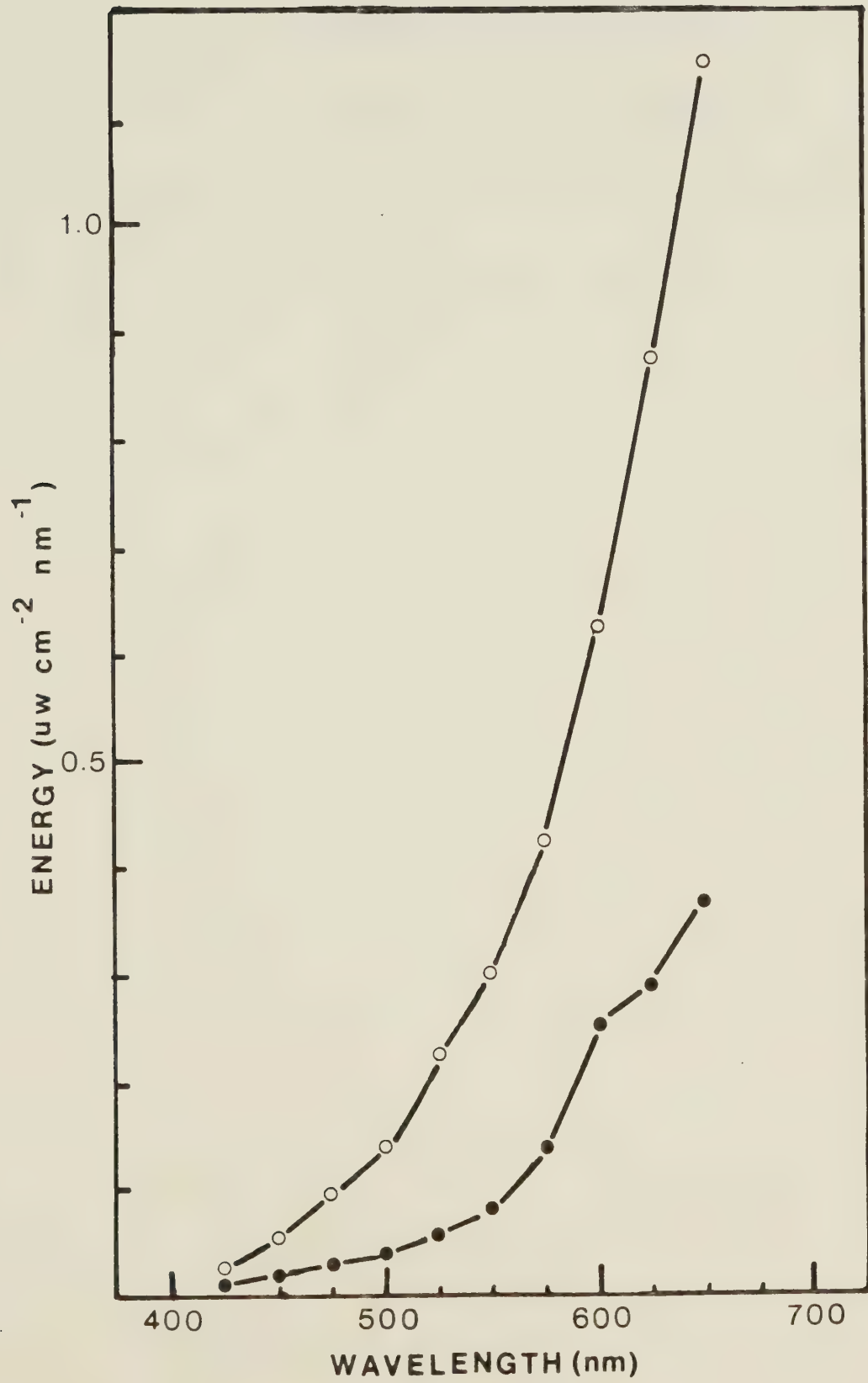
TABLE I. The 2-choice Discrete Operant Conditioning Paradigm

STIMULUS ▶ RESPONSE
                   ▼
           approach ▶ reinforcement
           or
           avoid   ▶ shock punishment

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of low motivational states and one other because of illness.

The first step involved shaping the behavior of the subjects for the acquisition of the conditioned response. This consisted of three tasks: approaching a broad band stimulus, stepwise reduction of luminance of the broad band light, and finally the introduction of monochromatic stimuli. The duration of the experiment for each group of fish was 15 days with four days of training (Days 1-4) (broad band stimuli), a one-day (Day 5) test to 90 percent criterion (broad band stimulus), and ten days of monochromatic testing (Days 6-15).

Muntz (1974) attributes the disparity in shape of photopic spectral sensitivity curves to the differences in the methodology employed by researchers. In light of this caution, explicit detail will be given to the specific steps in the training and testing protocol:

Day 1 — Each fish was given 60 trials in a no-choice situation in which positive reinforcement was administered on a partial schedule. A clear plexiglas barrier prevented entrance to the dark side but permitted viewing. The fish was manipulated (moved by a steel rod) into the response chamber and directed towards the stimulus for the first 10 - 20 trials. The frequent positive reinforcement usually provided the impetus for the subject to move on its own "initiative" at the 10 - 20 trial stage. The luminance of the broad band stimulus was 1.2 nit.

Day 2 — Each fish was given 15 trials in the no-choice situation and a subsequent 45 trials of a two-choice situation with access to either chamber. A correct response was positively reinforced (partial schedule) and an incorrect response resulted in negative reinforcement of two shocks (luminance 1.2 nit).

Day 3 — Each fish was given 60 two-choice trials (luminance 1.2 nit.

Day 4 — Each fish was given 60 two-choice trials, 30 trials at a luminance of 1.2 nit and 30 more trials at 0.52 nit.

Day 5 — Each fish was given a test to criterion. The criterion was set at 90 percent correct for a block of 10 trials with a stimulus set at a luminance of 0.52 nit.

Days 6-15 — Each fish was tested (threshold determination) at one wavelength per day for a total of ten wavelengths ranging from 425 to 650 nm in 25 nm increments. The threshold criterion was set at 75 percent correct response. Half of the fish in a group started the test at 425 nm on day 6 and ended with 650 nm on day 15 and the other half were tested in an opposite order of wavelengths.

The subject was given a 10-15 minute acclimation period followed by a 10 - 20 trial "warm-up" period at a supra-threshold (determined in pilot study) intensity (exitance) of a given wavelength. The test fish usually scored 80 - 90 percent correct during this period. Then the test proper commenced with 10 - 15 trials at an intensity which would elicit a response greater than 75 percent correct (10 trials if 90 percent was scored in the first 10 trials and 15 trials if the fish scored less than 90 percent in the first 10 trials), then 10 - 15 trials at an intensity expected to result in a score less than 75 percent correct. If, however, the step 2 score was not below 75 percent, another 10 - 15 trials at a lower intensity would be required to estimate the psychometric function (threshold).

The response latency was recorded for each trial. This measurement represented the duration from the time the gates were opened

simultaneously till the tail of the fish passed through the gate and into the proper response chamber.

Results

The psychometric functions for photopic spectral sensitivity of each fish were determined by linear interpolation. These estimates were corrected for the spectral transmission character of water (Hutchinson, 1957) within the test tank and then converted to photons (Goldsmith, 1973). Sensitivity was defined as the log of the reciprocal of the number of photons required to elicit a 75 percent response. The mean photopic sensitivity curve was calculated for each test wavelength and plotted as a function of wavelength.

Figure 4 illustrates three randomly chosen individual sensitivity curves and the mean sensitivity curve for all individuals. The peak of the mean sensitivity curve lies at 550 nm with a decrease of sensitivity towards the red and blue ends of the spectrum. There appeared to be three distinct components in this sensitivity function: from 425 - 500, 525 - 575, and 600 - 650 nm. The components in turn have peak sensitivities at 475, 550, and 625 nm, respectively. It is important to note that the mean sensitivity curve is based on data which have not been adjusted for differences in absolute sensitivity.

A mean sensitivity function can be misleading since less sensitive individuals make a proportionally greater contribution. A better method to estimate the shape of this photopic spectral sensitivity function would be to eliminate error due to differences in absolute sensitivity. This can be obtained by first normalizing each individual's results

Figure 4. Mean spectral sensitivity for 14 fish (o-●, bottom

curve) and individual spectral sensitivities (x-x,

fish 103; o-o fish 204; ●-●, fish 307). Note right

ordinate has the same label as left.
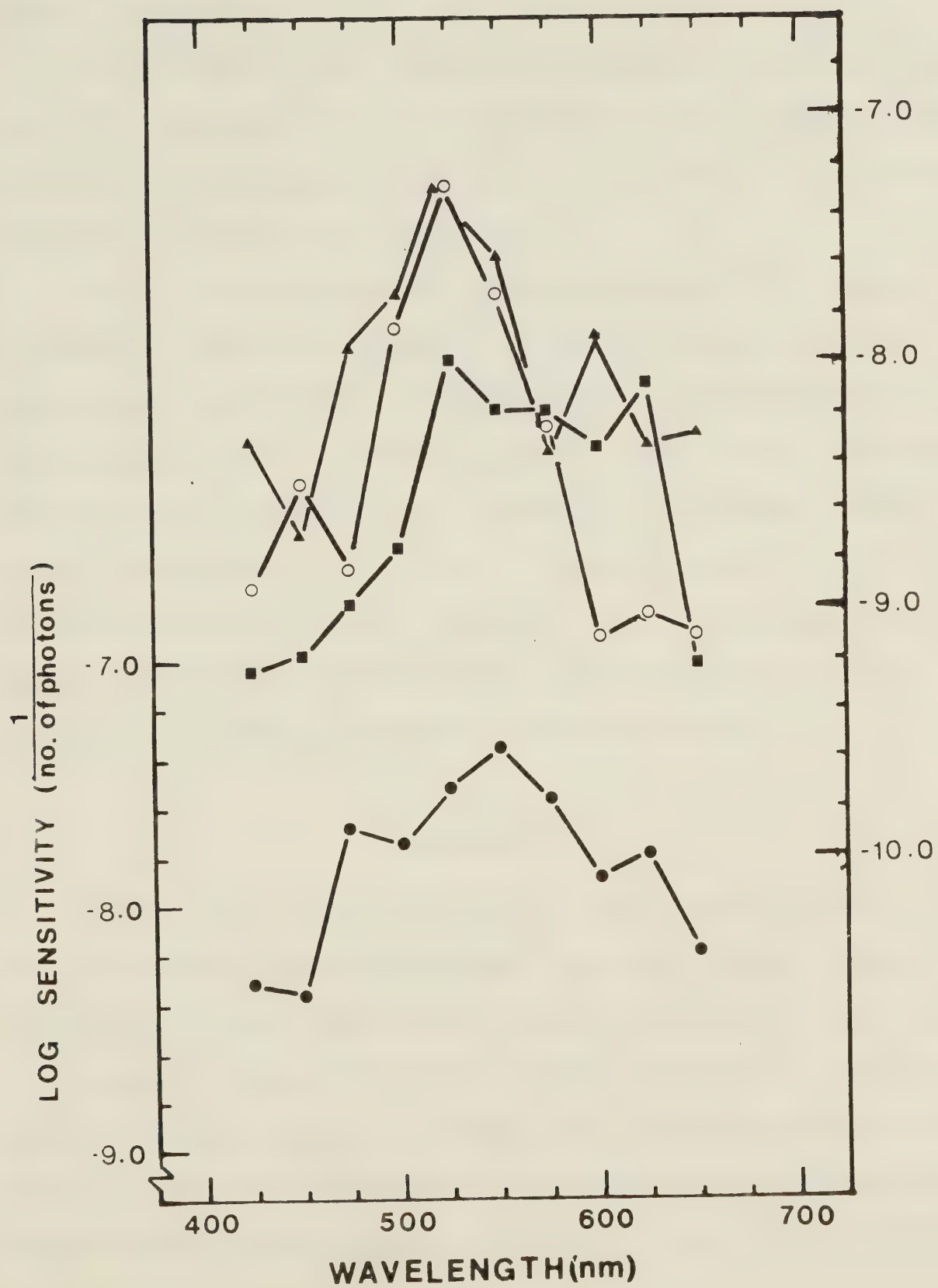
across wavelength, and then estimating the mean for all individuals on each wavelength. The standard error of this normalized data set represents variability in the shape of the photopic spectral sensitivities between individuals.

Mean normalized spectral sensitivity is shown in Figure 5. This curve also appeared to have three components similar to those revealed in Figure 4. Maximum normalized sensitivity occurred at 525 nm compared to 550 nm for maximum mean sensitivity.

Table II lists the mean and standard deviations of the response latencies for each test wavelength. A one-way analysis of variance demonstrated that the effect of wavelength on response latency was not significant ($p > 0.10$). Regression analysis indicated that absolute sensitivity was not significantly dependent on the response latency except at 525 nm ($p < 0.05$). Table III lists the number of trials to 90 percent criterion for each individual. There did not appear to be any noticeable effect of the number of trials to criterion on the absolute sensitivity and spectral sensitivity functions.

Discussion

Similar studies on goldfish (Yager, 1967; Beauchamp and Rowe, 1977) and on rudd (Muntz and Northmore, 1970) have yielded spectral sensitivity functions which differ somewhat from rainbow trout. The trout were less sensitive to short and long wavelengths. Perhaps rainbow trout possess chromatic mechanisms which differ from other species. However, conspecific and interspecific disparity in photopic spectral sensitivity may be due to subtle differences in test protocol, specific

Figure 5. Mean normalized spectral sensitivity with standard error

for 14 fish.
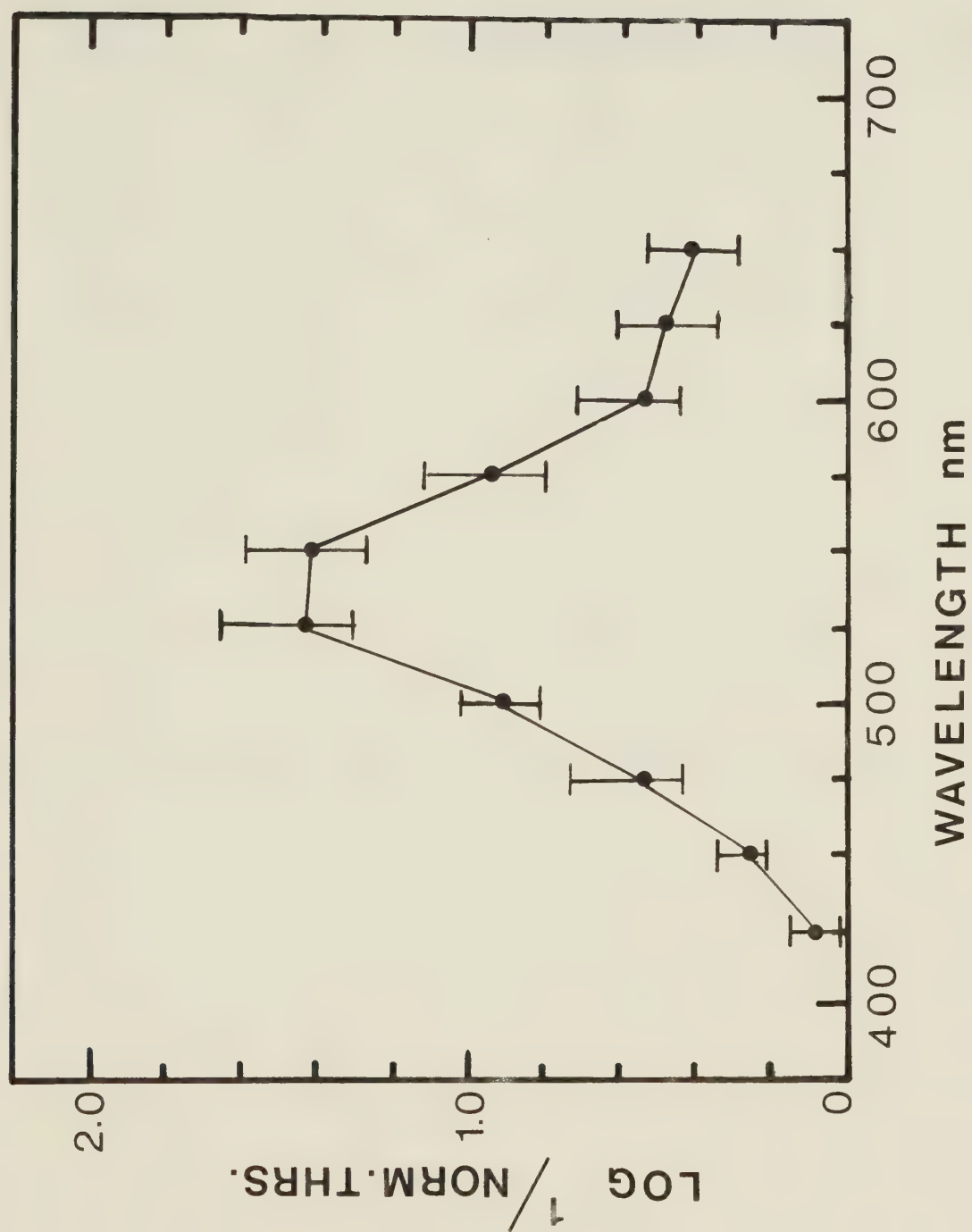
TABLE II. The Means and Standard Errors for the Response Latencies

of each Test Wavelength

Test Wavelength (mm)	Mean Response Latency (sec)	Standard Deviation
425	14.02	7.27
450	12.26	4.67
475	11.86	4.77
500	10.52	3.52
525	10.50	4.47
550	11.11	4.88
575	11.70	5.73
600	10.99	4.14
625	13.36	6.65
650	15.77	9.14

TABLE III. The Trials to 90 Percent Criterion for Each Test Subject

(Day 5)

Fish Number	Trials to 90% Criterion
101	10
102	10
103	20
104	10
105	10
201	10
202	60
203	40
204	10
301	20
302	10
303	50
305	10
307	10

test conditions, and the equipment employed (Muntz, 1974). For instance, the chromatic characteristics of a background adapting lamp could influence the shape of a photopic spectral sensitivity curve (Cronly-Dillion and Muntz, 1965; Yager, 1969; Muntz and Northmore, 1971). In our experiments, the overhead adapting light had a spectral energy distribution that consisted of predominantly long wavelengths. This potentially could reduce sensitivity at the red end of the spectrum through selective pigment bleaching. Concerning differences in method, our overhead adapting light was continuously present whereas Yager's (1967) was on only for 30 records between trials. Considering the different recovery rates of chromatic mechanism, this could interact with response latency to affect spectral sensitivity.

The demarcation of three curve components in Figures 4 and 5 is suggestive of a multiple receptive system with receptor interaction. Information concerning the characteristics of cone pigments and the neurophysiology of photopic vision in rainbow trout could facilitate the interpretation of a psychophysically determined photopic spectral sensitivity curve. Unfortunately, such physiological data do not seem to be available yet for this species.

Chapter III

THE TOXICITY AND TISSUE UPTAKE OF METHYLMERCURY

ADMINISTERED INTRAPERITONEALLY TO RAINBOW

TROUT (*SALMO GAIRDNERI* RICHARDSON)

Introduction

The acute toxicity of methylmercury to fish has been examined by
administration of methylmercury in the water (McKim et al., 1976);
oral injection (Miettinen et al., 1970); and intraperitoneal injections
(present study). McKim et al. (1976) found that the 96 hour LC50 for
yearling brook trout (102 g) was 65 μg Hg/ℓ. Miettinen et al. (1970)
found that the 30 day LD50 for MMC (methylmercuric chloride) adminis-
tered orally in three to four portions was 20 to 25 mg MMC/kg of body
weight.

An intraperitoneal injection was employed as the mode of toxicant
application in the present study since administration of MMC in food
(oral catheterization) or in water generally leads to a great deal of
between-animal variability in the rates and magnitude of MMC accumula-
tion. These differences in accumulation may be due to differences in
the quantity of food ingested, regurgitated, and MMC passed in the
feces; and to differences in the intensity of activity and the physical
parameters of the MMC test water. The use of an intraperitoneal
injection facilitates the examination of sublethal effects of metabo-
lizable toxicants such as methylmercury since one is able to reproduce
tissue levels of the toxicants which are similar to those found in some

natural populations. This then allows measurement of some parameter to be made at different tissue concentrations of the toxicant.

Different tissues possess varying capacities for the uptake and the elimination of methylmercury and show differences in the magnitude of pathological damage. For instance, Giblin and Massaro (1973) state that the brain of rainbow trout was found to accumulate and release methylmercury at a slower rate than other tissues probably because the blood brain barrier exerts some control over the passage of methyl-mercury. However, it has been reported that following long exposure, the Hg concentration in the brain may exceed that of the muscle (Miettinen et al., 1970; McKim et al., 1976). This may be a consequence of the high concentration of sulfhydryl containing compounds in the nervous system which bind irreversibly with methylmercury (Manalis and Cooper, 1975). The magnitude of neuropathological damage has also been shown to be highly correlated with regional differences in the concentration of selenium (Evans et al., 1977).

Many studies involving the neurotoxicity of methylmercury have stressed the importance of the latency of neurological symptoms subsequent to methylmercury exposure (Berlin et al., 1973; Evans et al., 1977). The duration of this latent phase is dependent on the frequency of methylmercury administration, size of the dose, and species of the test organism. Most studies of primates cite latencies of 20 to 50 days depending on the behavioral parameter measured.

The objective of the present study was to determine the 15 day LD50 for a single intraperitoneal dose of MMC and, secondly, to measure the concentration (tissue uptake) of Hg in the brain, eye, and epaxial

musculature 30 days following an injection of different sublethal doses of MMC.

Materials and Methods

Toxicity

Fish were kept in four flow-through, temperature controlled (15 ± 1°C) 20 ℓ aquaria. The test fish were obtained from Duggans Trout Farm (Namao, Alta.) and Sam Livingston Fish Hatchery (Calgary, Alta.). The size of the fish tested were from 10 to 20 cm (fork length). Each fish was anesthetized with 100 ppm MS222 (ethyl-m-amino benzoate methanesulfonate) then weighed and tagged. The fish were distributed to their respective tanks by stratified random assignment. They were given a one-week acclimation period before injection and fed Ewos brand pellets *ad libitum* daily. The methylmercuric chloride (MMC) solution consisted of MMC salt dissolved in a 0.02 M Na_2CO_3 solution and adjusted to a pH of 7.2 with 5 percent HNO_3 yielding a solution containing 2.57 mg of MMC per ml. The control solution was 0.02 M Na_2CO_3 adjusted to a pH of 7.2 with 5 percent HNO_3. The MMC was administered weight specifically, intraperitoneally, just anterior to the pelvic fins. The fish in the control group were injected with an array of injection volumes equivalent to those given to the experimental fish.

Three separate experiments were carried out to estimate the 15 day LD50. The protocol of these experiments is illustrated in Table IVa.

The time of death was recorded for each fish and the surviving fish were all sacrificed by placing them in a 1000 mg/ℓ solution of MS222

TABLE IV. Experimental Protocols for Toxicity and Tissue Uptake

Experiments

(a) Experimental Protocol and Percent Mortality in 15 Days

Experiment I	Dose	0^1	10^2	15	20
	n	6	6	6	6
	% mortality	0	83	100	100
Experiment II	Dose	0	2	5	8
	n	6	6	6	6
	% mortality	0	33	83	67
Experiment III	Dose	0	2	4	6
	n	9	8	8	8
	% mortality	0	0	13	50

(b) Experimental Protocol for Tissue Uptake Experiments

Experiment I	Dose	0	0.3^3	0.6	0.8
	n	6	6	6	6
Experiment II	Dose	0	0.3	0.6	0.8
	n	6	5	5	5

[1]Controls injected with saline at a similar array of injection volumes to those of the experimentals.

[2]All dose values are in mg of Hg/kg of body weight. Note that MMC was injected; however, the values are reported as Hg since 80 percent of MMC is Hg. Therefore 12.9 mg MMC/kg is equivalent to 10 mg Hg/kg.

[3]Toxic units corresponding to 0, 1.8, 3.6, 4.8 mg Hg/kg (0, 2.3, 4.6, 6.2 mg MMC).

on the fifteenth day of the experiment. The fish were then frozen and held at -30°C until total mercury analysis was performed.

Whole fish were wet ashed in preparation for total mercury analysis. Each individual was weighed and subsequently placed in 100 mls of concentrated H_2SO_4 and 50 mls of concentrated HNO_3 for 24 hours at room temperature. Three 3-ml aliquots of each digested fish were taken for total mercury analysis. Each aliquot was then oxidized by adding 7 mls of 7 percent $KMNO_4$ and reduced by 0.1 mls of a 1 percent solution of $SnCl_2$. The analysis for total mercury content in digested samples utilized a flameless atomic absorption spectrophotometer (Unicam SP90A Spectrophotometer) (Armstrong and Uthe, 1971; Munson, 1978).

A standard curve was estimated for each set of samples of fish, using a mercuric chloride standard (absorbance versus µg Hg). Chemical blanks were used to test for background concentrations of Hg.

A recovery of 87 to 102 percent of the total mercury present was determined by recovery experiments using samples containing known amounts of $HgCl_2$ and CH_3HgCl. Accuracy of the analysis was also monitored by measuring the total Hg content of standard orchard leaves, 0.147 ± 0.056 S.D. µg/g (n = 24) standard reference material 1571. The Hg levels obtained were 95 percent of the values reported (0.155 ± 0.015 µg/g) for these leaves (Munson, 1978).

Tissue Uptake of Methylmercury

Two experiments were employed to examine the effect of dose on Hg concentration in tissues and to determine the difference in Hg uptake between tissues, 30 days after the fish were injected intraperitoneally. The fish were obtained from Sam Livingston Fish Hatchery (Calgary, Alta.)

and ranged in size from 10 to 20 cm in fork length. All fish for a given experiment were placed together in a 200 ℓ continuous flow-through tank and allowed one week acclimation at 15 ± -.1°C. The fish were fed Ewos brand pellets *ad libitum* daily.

The fish were divided into four dose classes: control, 0.3, 0.6, 0.8 toxic units. The dose equivalent to one toxic unit was taken to be the 15 day LD50 which had previously been determined. The corresponding dose levels were: 0, 1.8, 3.6, 4.8 mg Hg (0, 2.3, 4.6, 6.2 mg MMC) per kg of body weight.

The experimental protocol is given in Table IVb. Each fish was anesthetized, weighed, tagged, injected intraperitoneally and then returned to the holding tank. The injection solutions were identical to those utilized in the toxicity test.

Fish were sacrificed on day 30 and the tissue samples were removed, placed in glass containers and frozen at -30°C. Total mercury analysis of these samples was identical to that for whole fish except the smaller quantities of tissue were digested in 2 mls of concentrated H_2SO_4 and 1 ml of concentrated HNO_3. Total mercury was expressed in µg Hg/g of wet tissue weight.

Results

Toxicity

Figure 6 and Table IVa illustrate the percent mortality 15 days after injection as a function of the dose of methylmercury administered. A regression analysis was performed in the 2 to 10 mg range of the independent variable to minimize error in the prediction of the 15 day

Figure 6. Percent mortality versus the dose administered (mg Hg/kg
of body weight). A regression analysis was performed on
2 to 10 mg range of independent variable and the equation
is $Y_i = 0 + 8.7(X_i)$.
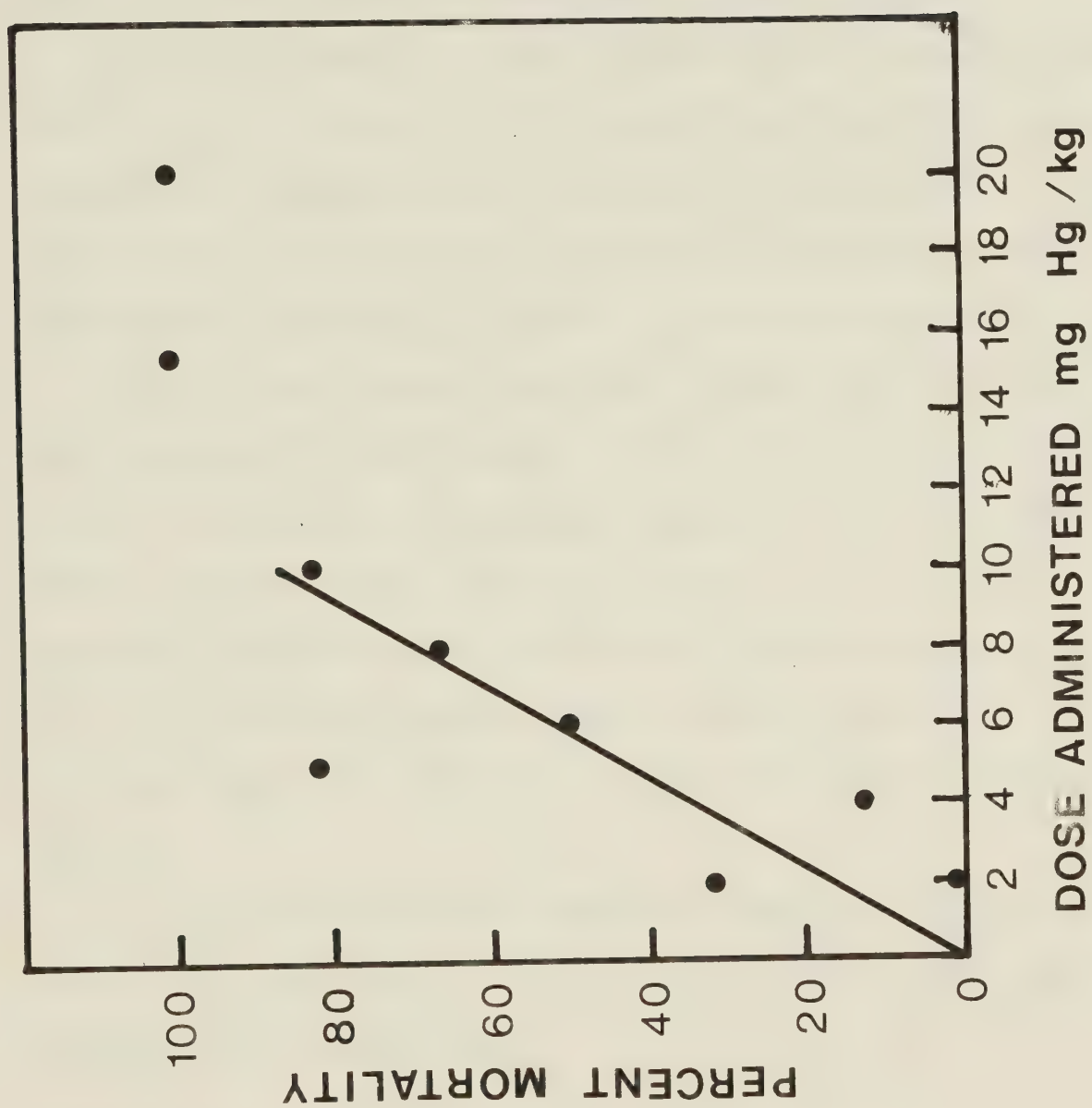
LD50 (10 mg was chosen as a maximum dose for the regression since curve inflection occurs at this level of the independent variable). The y-intercept was not significantly different ($p > 0.01$) from the origin, therefore a line was fitted to pass through the origin and the $\bar{X}$, $\bar{Y}$ point for the 2 to 10 mg range. The standard error of this line was 4.1 and the regression coefficient (slope) was 8.7.

The 15 day LD50 for a single intraperitoneal injection in rainbow trout is 5.7 ± 0.4 mg Hg (7.1 ± 0.4 mg MMC) per kg of body weight, determined by interpolation. The mean uptake (body burden) for this was 5.1 ± 0.6 μg Hg/g of body tissue (Fig. 7) indicating that the fish accumulated approximately 90 percent of the dose administered.

The fish from toxicity experiments two and three (Table IVa) were used to measure the body burden of Hg (Fig. 7). A one-way analysis of variance demonstrated a significant difference in the mean uptake between the various dose classes ($p < 0.001$). The y-intercept of the regression equation was not significantly different from origin ($p < 0.01$) and thus a straight line was fitted through 0 and $\bar{X}$, $\bar{Y}$ yielding a regression coefficient of 0.91 and a standard error of 0.11 (Fig. 7).

For the remainder of this paper the 15 day LD50 will be referred to as 1 toxic unit (tu) and any dose less than 1 toxic unit will be considered as a sublethal dose.

Tissue Uptake

A one-way analysis of variance indicated a significant difference between the mercury content of the three tissues tested (brain > muscle > eye) (all $p < 0.001$) for each dose level: 0.3, 0.6, 0.8 tu (Fig. 8).

Figure 7. Body burden or the whole fish mercury content (µg Hg/g
           of tissue) versus the dose administered (mg Hg/kg of body
           weight). The regression equation is Yi = 0 + 0.91(Xi).

DOSE ADMINISTERED mg Hg /kg

BODY BURDEN ug Hg/g of tissue
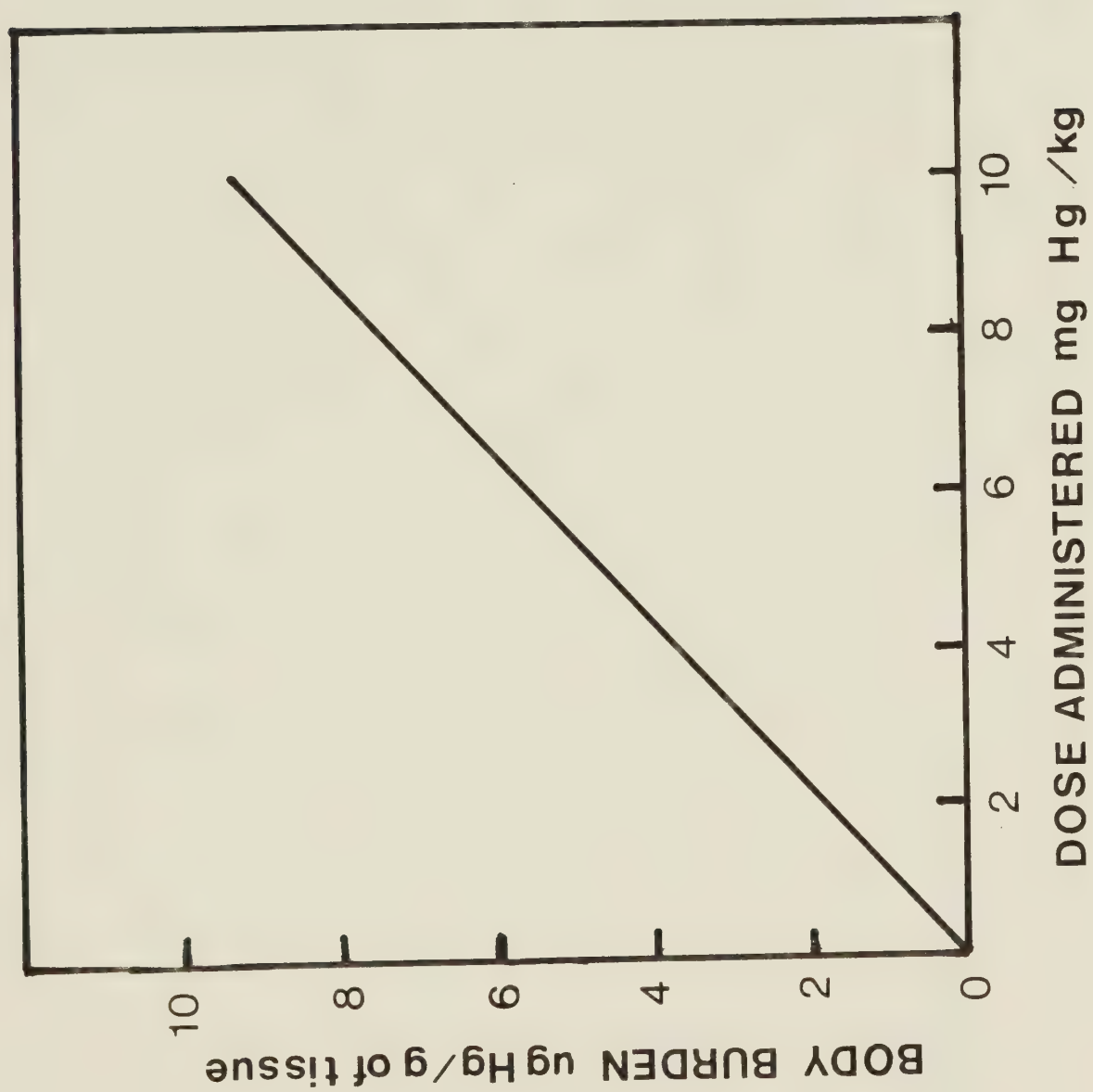
Figure 8. Tissue concentration; brain, muscle, eye; of mercury

(μg Hg/g of tissue) versus the dose administered (toxic

units: 0.3, 0.6, 0.8). The respective regression

equations are: $Y_i = 0 + 4.76(X_i)$, $Y_i = 0 + 3.91(X_i)$,

$Y_i = 0 + 2.3(X_i)$.
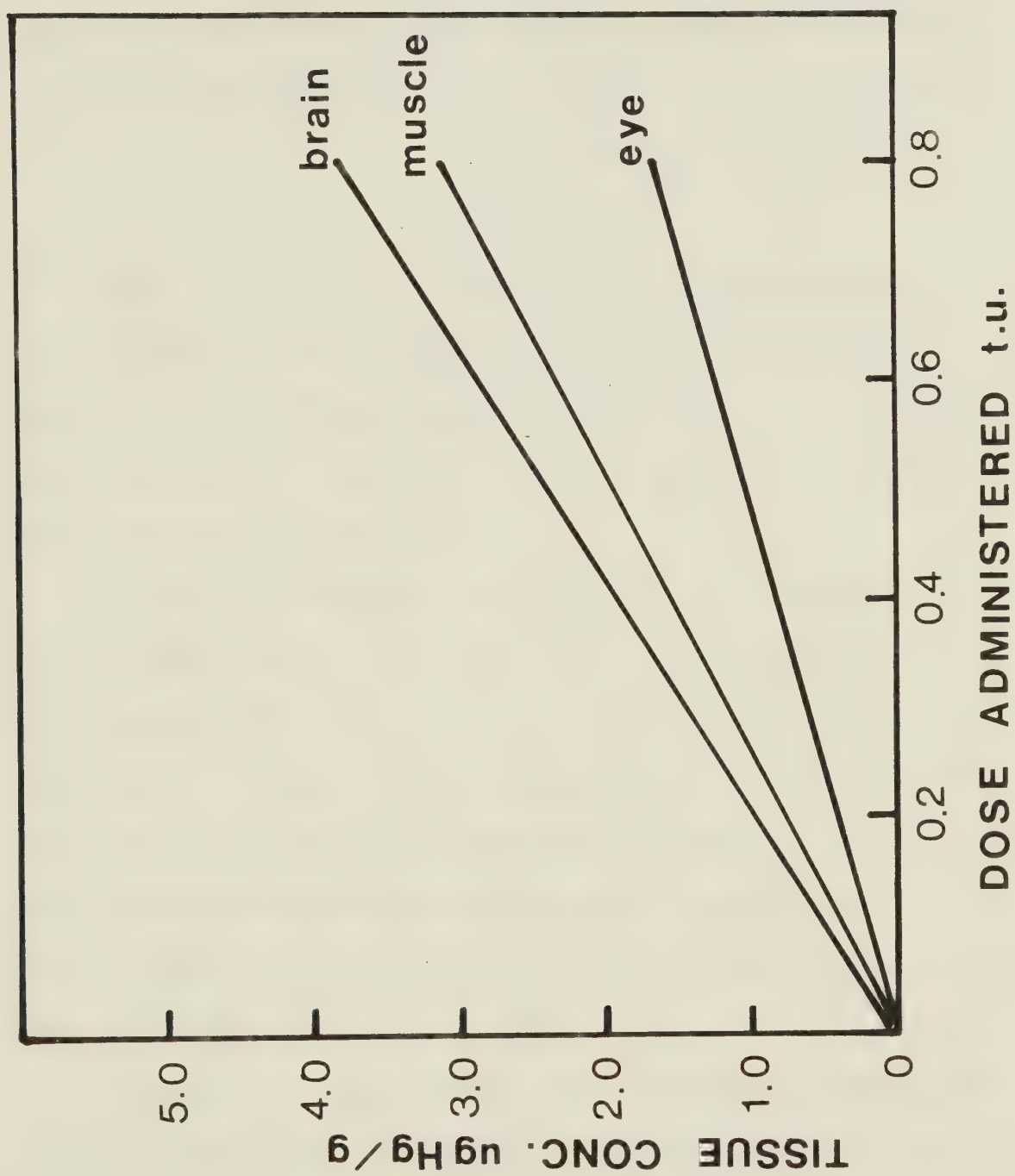
The concentration of mercury in each tissue increased significantly (p < 0.001) with increasing sublethal dose of methylmercury.

The y-intercepts for the regression equations of all three tissues were not significantly different from origin (all p > 0.01) and therefore a line was fitted to pass through the origin and the regression coefficients and the standard errors are as follows: 4.76 ± 0.49 (brain); 3.91 ± 0.39 (muscle); 2.03 ± 0.26 (eye).

Discussion

Acute toxicity studies dealing with the intraperitoneal or oral mode of administration of methylmercury have been shown to be influenced by the rate of application of the toxicant. This, unfortunately, makes it difficult to establish standard methods and therefore reduces the comparability of results.

The LD50 of the present study and that of Miettinen et al. (1970) differ, unequivocally. Miettinen et al. (1970) applied 9.6 to 19 mg methylmercury per kg body weight in three or four portions with a two day interval, whereas I used a single dose of 2 to 10 mg Hg/kg of body weight. An 8 or 10 mg Hg/kg dose was highly lethal in my experiment whereas this same dose administered in several portions over a period of days resulted in survival beyond the thirtieth day of the test period (Miettinen et al., 1970).

It appears that the retention of methylmercury differs with respect to the method of application. Intraperitoneal injections resulted in retention of 90 percent of the dose administered; on the other hand, only 53 percent of the dose administered orally was

retained (Miettinen et al., 1970). Once methylmercury is in the circulatory system, elimination occurs through both the feces and urine regardless of the mode of administration (Munson, 1978).

The duration of a toxicity test is an important consideration when designing acute toxicity experiments of this nature. The mode of toxicity of methylmercury associated with chronic exposure to MMC appears to be via pathological changes in the nervous system (Bäckström, 1969; Miyakawa et al., 1970; Berlin et al., 1973; Manalis and Cooper, 1975; Evans et al., 1977). A reasonably long period of chronic exposure is necessary for methylmercury to accumulate to levels which inflict neuropathological damage. Moreover, this latency between injection and the onset of behavioral aberrations seems to be dependent on the volume and frequency of dosing as well as species specificity (Evans et al., 1977).

McKim et al. (1976) report that the concentration of MMC in any given tissue at any time following exposure is dependent on the concentration of MMC in the water. My results support this contention indirectly since I was able to demonstrate a difference in the uptake of Hg between the dose categories within each tissue. In fact the relationship showed a significant ($p < 0.001$) regression for each tissue.

Several laboratory studies have indicated that brain concentrations of mercury exceed epaxial musculature concentrations. However, this is by no means a consistent trend for all species of fish. The difference in magnitude of uptake between tissues in rainbow trout is illustrated in Figure 8. The high brain levels could be explained by preferential

accumulation in nervous tissue or by a slow turnover of methylmercury in the brain compared with other tissues. After 30 days the other tissues had lost most of their accumulated methylmercury but the brain had not. The chemical nature of the nervous system could influence the mobility of methylmercury (Manalis and Cooper, 1975).

The relationship between body concentrations of Hg and dose does not differ from that found for muscle concentration of Hg (slope test $p > 0.05$) and dose in this study. This has also been shown by McKim et al. (1976) and by Miettinen et al. (1970) and they point out that this is obviously so since the muscle tissue represents the largest component of the body.

The small variance in the dose-response relationship which was found in the three tissues examined indicates that intraperitoneal administration may be particularly suitable for behavioral or physio-logical studies dealing with sublethal doses of methylmercury. In addition, I was able to produce some tissue levels of mercury which are near those that are commonly found in areas of mercury contamination.

Chapter IV

COLOR VISION DEFICIENCIES AS A CONSEQUENCE OF

METHYLMERCURY EXPOSURE IN RAINBOW TROUT

Introduction

In the past, most research on the effects of methylmercury has been concentrated on mammals, chiefly humans. As a consequence there are very few direct demonstrations of the harm inflicted on fish due to methylmercury exposure. One must consider the effects of mercury on fish physiology and behavior to comment on the long-term contribution of fish to the ecosystem and their future availability as food for man.

Behavior has been utilized to assess the impact of toxicants in the past. Open field behavior was used to assess the effect of methylmercury on mice (Suzuki, 1969; Spyker et al., 1972); preference and avoidance reactions (Sprague, 1968; Scherer, 1975), and open field orientation (Kleerekoper et al., 1972) were utilized to assess the effects of heavy metals in fish. Operant conditioning techniques were used to evaluate pesticides (McNicholl, 1974) and heavy metals (Weir and Hine, 1970) in fish. While rainbow trout are recognized as the standard test species, little is known about the effect of methylmercury on their behavior.

The toxicity of methylmercury in primates involves neuropathological changes which are particularly notable in the visual system (Evans et al., 1977). It appears that the regional differences in the uptake of methylmercury in the brain selectively lesions the lateral

geniculate nucleus and the visual and motor cortexes in primates. As a consequence, one observed the development of behavioral symptoms such as a reduced sensitivity to visual stimuli of a low luminance, constriction of visual fields and a reduction in shape and pattern discrimination.

The fact that methylmercury inflicts damage on the visual system and that abnormalities in color vision result from such neuropathological conditions in humans (Lyle, 1976) led to the examination of the effect of methylmercury on the color vision of rainbow trout. The effect of sublethal exposure to methylmercury on the photopic spectral sensitivity of rainbow trout was measured for 10 wavelengths between 425 and 650 nm using a behavioral forced choice task.

Materials and Methods

Protocol

The experimental procedure consisted of three 15-day stages. During the first stage 15 rainbow trout were trained to discriminate between the presence and absence of a spectrally neutral stimulus in a two-choice shuttle tank with reinforcement (beef heart) and punishment (shock) contingencies (Days 1-4) (see Chapter II). On Day 5, these individuals were given a test of trials to 90 percent criterion based on blocks of 10 trials. Then their spectral sensitivities were determined at ten wavelengths from 425 to 650 nm (Days 6-15). The second phase (Days 16-30) involved the administration of weight-specific injections of a control solution or a methylmercuric chloride solution corresponding to the toxic unit values of 0.0 (control saline, n = 4),

0.3 (n = 2), 0.6 (n = 4), and 0.8 (n = 5). One toxic unit is equivalent to a dose of 5.7 mg Hg/kg of body weight (see Chapter III). After injection the fish were held for 15 days to allow time for the methylmercury to be distributed in the body, a process which results in a concentrated uptake by brain tissue. The fish were then fed beef heart *ad libitum* daily. The third phase represented a retest period (Days 31-45) and was virtually identical to phase I with exception of the Days 31 to 35 period. On Day 31 the fish were given a test to criterion similar to the test given on Day 5. If an individual required a greater number of trials to reach criterion in phase III than in phase I it was trained to its original level of performance, during Days 32 to 35. Days 36 to 45 involved wavelength testing identical to phase I spectral sensitivity determinations.

Results

The effect of methylmercury on photopic spectral sensitivity was examined in two ways: one comparing the absolute sensitivity of the controls and experimentals in the post exposure phase, the other employing ratios of phase I absolute sensitivity to phase III absolute sensitivity for the control and experimental fish.

Average spectral sensitivity as a function of wavelength is shown for the control, 0.6, and 0.8 dosage groups during the pre (Fig. 9a) and post (Fig. 9b) treatment test (the 0.3. toxic unit group was excluded since it had only two individuals). Figure 9a indicates there was little difference in the average photopic spectral sensitivity of the individuals in these three treatment groups prior to methylmercury

Figure 9. Log mean absolute photopic spectral sensitivity of the control (o-o), 0.6 (Δ-Δ), and 0.8 (●-●) toxic unit groups (a) prior to methylmercury exposure (there were no significant differences between these three groups) and (b) post methylmercury exposure (there are no significant differences between the control and 0.6 t.u. group, but the control and 0.8 t.u. are significantly different at 475, 500, 525, and 600 nm) (control and 0.6 t.u. groups n=4, 0.8 t.u. group n=5).
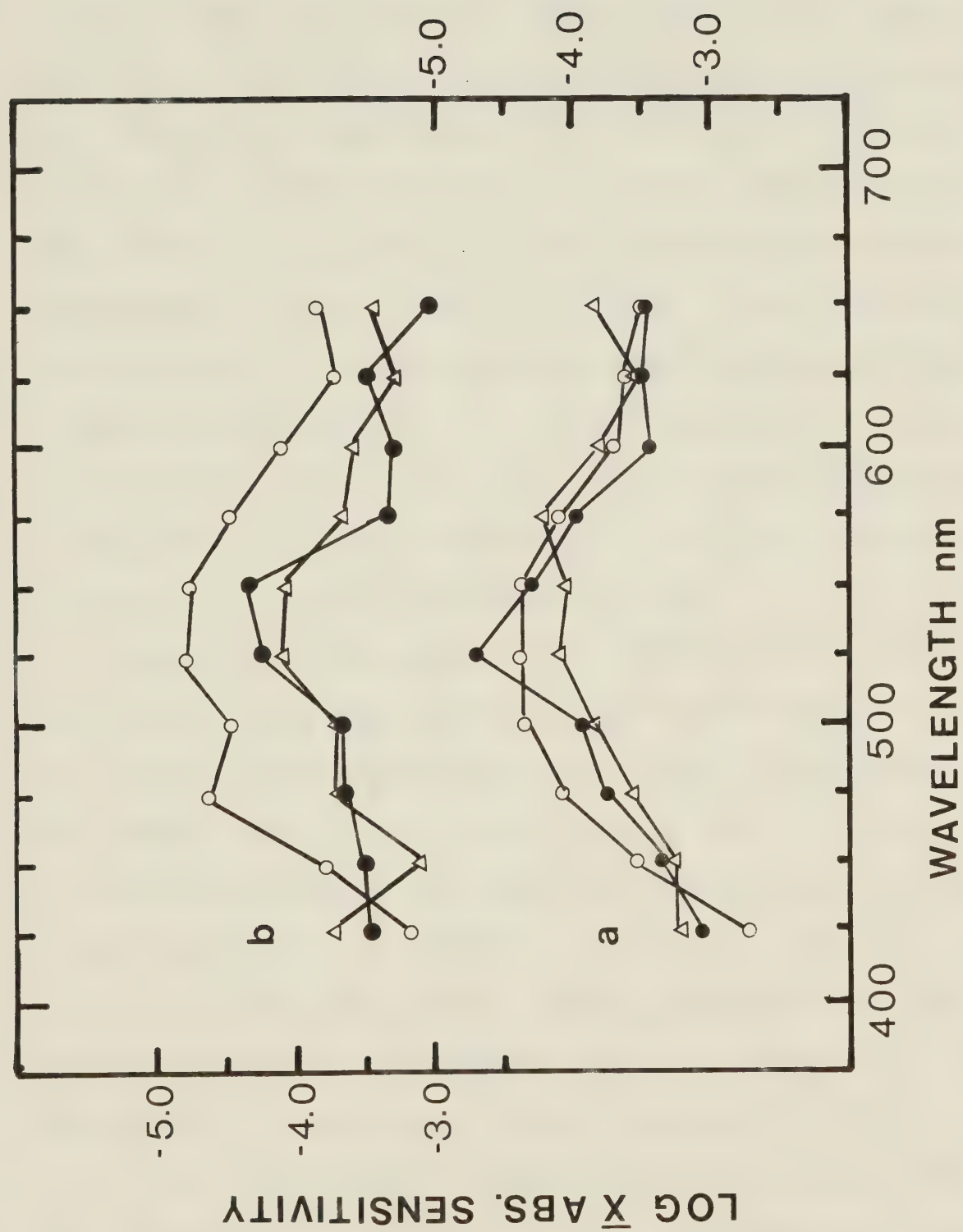
treatment. One-way analysis of variance indicated that none of the differences in sensitivity are significant ($p > 0.05$ for all wavelengths). More specifically, Student t-tests of the control versus 0.6 t.u. (toxic unit) group; the control versus 0.8 t.u. group; and the 0.6 t.u. group versus the 0.8 t.u. group were all nonsignificant ($p > 0.05$) for all wavelengths. After methylmercury exposure, however, the analysis of variance for each wavelength demonstrated that the controls, 0.6, and 0.8 t.u. groups had significantly different absolute sensitivities at 525 nm ($p < 0.05$). Student t-test of the control versus 0.8 for each wavelength showed significant differences in absolute sensitivity at 475 nm ($p < 0.05$), 500 nm ($p < 0.05$), 525 nm ($p < 0.01$) and 600 nm ($p < 0.05$). Student t-tests comparing the control and 0.6 t.u. groups and the 0.6 and 0.8 t.u. groups for each wavelength were all nonsignificant ($p > 0.05$).

The ratio of pre to post treatment absolute sensitivity for each fish can more accurately represent the treatment effects since individual fish can differ considerably in sensitivity of discrimination and the number of fish within a dosage group was small. In Figure 10 the mean visual performance ratio was plotted for each treatment group (excluding 0.3 t.u.) as a function of wavelength. While the control group demonstrated some expected overall improvement in photopic spectral sensitivity, the methylmercury-treated groups showed less improvement or, in some cases, actual decrements in sensitivity.

Table 5 indicates that methylmercury has its largest effect on the sensitivity at 525, 600, and 650 nm. The partial correlation coefficients and the one-way analysis of variance were significant

Figure 10.   Log mean visual performance ratios for the control (o-o),

0.6 (Δ-Δ), and 0.8 (●-●) toxic unit groups. The visual

performance ratios represent a change in the energy

required to reach the 75 percent threshold at a specific

test wavelength, from phase I to phase III (pre to post

methylmercury exposure). Refer to Table I for statistical

tests (control and 0.6 t.u. groups n=4, 0.8 t.u. group

n=5).
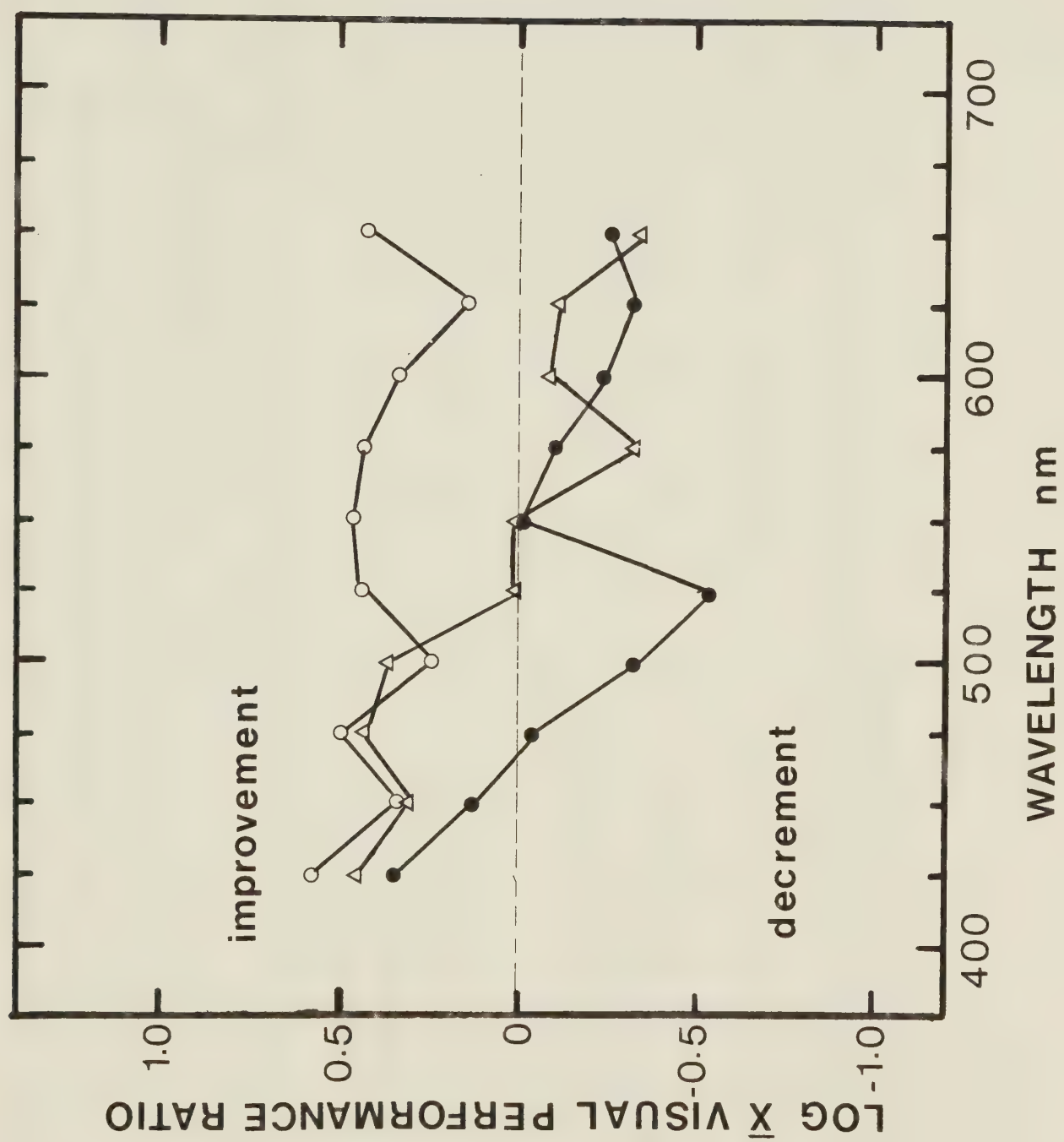
TABLE V. Results of Wavelength Specific Statistical Tests Performed to Test for Significant Differences in Mean Visual Performance Ratios Between Treatment Groups

Statistical Test	Wave Length (nm)									
	425	450	475	500	525	550	575	600	625	650
Partial correlation coefficients										
visual performance ratio vs. dose[1]	-0.42	-0.15	-0.33	-0.21	-0.82	-0.61	-0.67	-0.81	-0.54	-0.78
Significance of r										
Hypothesis Ho:ρ=0	NS[2]	NS	NS	NS	**	*	*	**	NS	**
One-way analysis of variance										
control vs. 0.6 t.u. vs. 0.8 t.u.	NS	NS	NS	*[3]	**[4]	*	*	**	NS	**

[1]The correlation coefficients were based on dose group control, 0.3, 0.6, 0.8. Note that the sample sizes of control and 0.6 group were 4 whereas that of 0.3 t.u. group was 2 and 0.8 group was 5. The 0.3 t.u. group was not used in ANOVA.

[2](p > 0.05)

[3](0.05 > p > 0.01)

[4](p < 0.01)

(p < 0.01) at these wavelengths. These findings generally support those illustrated in Figure 9b.

A one-way analysis of variance indicated that ratio of pre to post treatment response latencies did not differ significantly between the control, 0.6, 0.8 t.u. group at any wavelength (p > 0.05). In addition an analysis of variance showed that ratios of pre to post treatment trials to criterion did not differ significantly between the control, 0.6 and 0.8 t.u. groups.

Discussion

The present study indicates that methylmercury impaired the ability of rainbow trout to discriminate spectral stimuli. There were no significant effects of methylmercury on the response latency; this indicates that methylmercury did not affect motivation. Also, methylmercury did not have a significant effect on the number of trials required to reach criterion; hence methylmercury appeared not to affect learning or memory. Some studies have demonstrated that learning and motivation do suffer impairment. Post et al. (1973) found that rats required more trials to learn a T-maze subsequent to oral administration of methylmercury. Berlin et al. (1973) found that low levels of methylmercury did not affect the response latency of monkeys, though high levels which produced severe visual symptoms lengthened latency due to difficulty in bringing the stimuli into view. The general lack of motivation and learning affects in the present study may therefore be attributed to the use of relatively low dosage levels. My results

suggest that the observed behavioral affects were primarily due to the effect of methylmercury on the visual system.

Photopic spectral sensitivity of the normal rainbow trout suggests three response components: one between 425 and 500 nm, another between 525 and 575 nm, and another between 600 and 650 nm. After methylmercury exposure the chromatic visual system appears to have the most difficulty detecting wavelengths which are intermediate to the three curve components. However, methylmercury treatment did not significantly affect sensitivity at the peak wavelengths of these components. If one examines the ratios in absolute sensitivity from pre to post methylmercury exposure, there is a similar trend. That is, the intermediate wavelengths suffer the most detriment and the peak wavelengths of the curve components show slight differences from the control. These results suggest that methylmercury exerts most of its influence on the post receptor neural processing of spectral information.

The color red plays an important role in the reproductive behavior of male rainbow trout and sticklebacks and any change in red sensitivity of these fishes could reduce the organism's reproductive success. Considering that color vision undoubtedly is important for various behaviors in trout, even moderate levels of methylmercury could reduce the organism's viability. The levels we employed have been commonly reported in natural populations of fish which were exposed to mercury (Miettinen et al., 1970).

Chapter V

GENERAL DISCUSSION

The results of this study suggest that rainbow trout have tri-chromatic color vision and that retinal interaction is responsible for the detection of certain wavelengths. The evidence for trichromacy was furnished by the psychophysical determination of photopic spectral sensitivity and from the effects of methylmercury on psychophysical photopic spectral sensitivity. The "normal" sensitivity curve may be characterized by three curve components with presumptive peak sensitiv-ities of 475, 550, and 625 nm. Unfortunately, there is no specific information with respect to the absorption spectra of photopic pigments or neurophysiology of the rainbow trout visual system.

Ali (1974) reports three anatomical cone types in brook trout (*Salvelinus fontinalis*); the stubby barrel-like cones of the dorsal retina were classified as blue-sensitive cones, the long, slender cones of the ventral region were classified as red-sensitive cones, and the mid retinal region had a mixture of double cones which appear to be made up of green- and red-sensitive cones. Given the fact that rainbow trout and brook trout are different species, they share a similar habitat and thus one should be able to speculate that they share a similar visual system. Tsin (personal communication) has indicated that rainbow trout possess a duplex retina and therefore my inference is supported.

I have observed some striking changes in photopic sensitivity which occurred subsequent to methylmercury exposure. These impairments in photopic sensitivity suggest that the neural processes of receptor

interaction are being affected by methylmercury. This premise was derived from observations which indicated a reduction in sensitivity at wavelengths that would seem to be mediated through retinal interaction such as blue green (500 nm) and orange (600 nm) wavelengths.

The nature of this interaction is unknown; whether it is inhibitory or additive is not clear. Some researchers indicate that local minima (troughs) in spectral sensitivity curves result when two receptor types of an opponent pair are equally stimulated, thus resulting in the greatest degree of mutual inhibition, at, for instance, a test stimuli of 600 nm (Muntz, 1974). On the other hand, these troughs may be formed by the partial additive response of two receptor types at low intensity of stimulation and further increases in intensity would smooth out the troughs. If the neural processes controlling this additive interaction were selectively affected by methylmercury, one would expect even lower sensitivities at wavelengths that are perceived through the interaction of receptors. Or perhaps methylmercury can evoke transmitter output, thus enhancing retinal inhibition between opponent pairs, such as Manalis and Cooper (1975) observed in frog neuromuscular preparations. Future experimentation in this area should include studies in which methylmercury exposure is localized through selective lesioning techniques. It is noteworthy that this study not only has indicated that methylmercury does damage the chromatic visual system but also points out that methylmercury can be used as a pharmacological tool for examining neural activity governing the visual system.

Many of the previous behavioral toxicology studies dealing with methylmercury have employed unusually high tissue concentrations of

mercury. It is not uncommon to see values of 20 to 50 µg Hg/g of tissue reported in the literature (Suzuki, 1969; Berlin et al., 1973; Evans et al., 1975). My study, however, uses tissue concentrations comparable to those found in natural populations of fish, such as 1.8 to 4.8 µg Hg/g of tissue (see Miettinen et al., 1970 for tissue concentrations). Thus many populations of fish may be encountering visual deficiencies induced by methylmercury contamination which could affect visually dependent behaviors such as predation, predator evasion, reproductive activity, and intra- and interspecific territoriality.

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APPENDICES

APPENDIX Ia

MEAN INTENSITY THRESHOLDS (E75%) AND MEAN RESPONSE LATENCIES FOR PHASES I AND III

TEST WAVELENGTH	n	TREATMENT GROUP	THRESHOLD ($\mu w\ cm^{-2}nm^{-1}$) Phase I $\bar{x}$	S.D.	Phase III $\bar{x}$	S.D.	RESPONSE LATENCY (sec) Phase I $\bar{x}$	S.D.	Phase III $\bar{x}$	S.D.
425	4	Control	0.00176	0.00186	0.000665	0.0009	12.93	8.33	13.55	6.40
	2	0.3 t.u.*	0.000715	0.000191	0.000218	0.000187	20.05	12.23	13.10	4.10
	4	0.6 t.u.	0.000555	0.000396	0.000191	0.0000982	13.78	8.00	13.35	9.21
	5	0.8 t.u.	0.000855	0.00104	0.000336	0.000295	12.68	5.10	12.96	3.56
450	4	Control	0.00290	0.000135	0.000167	0.000102	12.88	6.25	13.35	3.56
	2	0.3 t.u.	0.000475	0.000304	0.000965	0.00118	14.4	6.08	16.50	8.49
	4	0.6 t.u.	0.00044	0.000242	0.000786	0.00115	10.75	3.78	12.65	3.48
	5	0.8 t.u.	0.000418	0.000321	0.000315	0.000195	12.12	4.71	11.72	5.51
475	4	Control	0.0000838	0.0000631	0.0000265	0.0000125	10.15	4.96	12.78	2.65
	2	0.3 t.u.	0.000238	0.000229	0.00029	0.000227	15.4	2.40	15.70	5.79
	4	0.6 t.u.	0.000272	0.000175	0.000204	0.000209	11.38	5.41	10.18	2.41
	5	0.8 t.u.	0.000170	0.000102	0.000269	0.000207	12.20	5.32	8.42	3.95
500	4	Control	0.0000448	0.0000197	0.000032	0.0000251	10.80	4.59	9.65	3.48
	3	0.3 t.u.	0.000174	0.0000969	0.000281	0.000172	13.03	3.49	18.43	10.19
	4	0.6 t.u.	0.000124	0.0000192	0.000224	0.00037	10.33	2.43	6.88	2.83
	5	0.8 t.u.	0.000100	0.0000697	0.000199	0.000124	8.94	3.48	10.96	5.19
525	4	Control	0.0000408	0.0000188	0.0000156	0.00000645	10.30	2.92	12.58	4.07
	3	0.3 t.u.	0.0000696	0.0000293	0.000068	0.0000261	15.43	3.73	17.10	7.07
	4	0.6 t.u.	0.0000708	0.0000575	0.0000748	0.0000469	9.63	5.59	9.15	1.95
	5	0.8 t.u.	0.0000187	0.0000193	0.000064	0.0000183	8.40	3.69	10.28	3.85
550	4	Control	0.0000413	0.0000354	0.0000179	0.000019	11.00	5.65	9.45	0.91
	3	0.3 t.u.	0.0000435	0.0000304	0.0000347	0.0000102	17.47	2.33	12.77	7.57
	4	0.6 t.u.	0.0000825	0.0000629	0.0000782	0.0000642	8.97	2.56	8.00	6.00
	5	0.8 t.u.	0.0000435	0.000040	0.0000454	0.0000313	9.08	4.19	9.20	2.63
575	4	Control	0.0000835	0.0000661	0.0000333	0.0000300	13.88	8.69	10.97	3.26
	3	0.3 t.u.	0.000140	0.000165	0.000073	0.0000398	13.30	5.86	10.73	2.51
	4	0.6 t.u.	0.0000530	0.0000196	0.000233	0.000227	8.25	3.35	5.73	2.56
	5	0.8 t.u.	0.000101	0.0000637	0.000432	0.000712	11.76	4.71	11.38	4.71
600	4	Control	0.000191	0.000144	0.0000847	0.0000477	10.45	3.64	13.33	5.71
	3	0.3 t.u.	0.000207	0.000113	0.000314	0.000249	11.03	4.73	11.10	7.20
	4	0.6 t.u.	0.000146	0.000129	0.000265	0.000249	11.43	5.06	8.10	5.08
	5	0.8 t.u.	0.000349	0.000367	0.000516	0.000413	11.06	4.83	13.92	9.23
625	4	Control	0.000263	0.00024	0.000186	0.000144	14.48	9.78	11.53	0.89
	3	0.3 t.u.	0.000154	0.000214	0.000163	0.0000907	13.73	8.13	12.86	8.00
	4	0.6 t.u.	0.000252	0.000249	0.000519	0.000514	12.08	6.95	11.25	5.85
	5	0.8 t.u.	0.00033	0.000392	0.00122	0.00131	13.30	4.66	12.14	8.30
650	4	Control	0.000298	0.000246	0.000136	0.000131	17.98	8.45	11.30	3.15
	3	0.3 t.u.	0.000412	0.000308	0.000217	0.000171	13.53	2.38	12.70	8.70
	4	0.6 t.u.	0.000105	0.0000049	0.000385	0.000267	10.33	3.33	9.80	1.64
	5	0.8 t.u.	0.000296	0.000217	0.000818	0.000956	19.70	13.33	15.82	14.79

*t.u. - toxic units

APPENDIX Ib

MEAN WHOLE BODY MERCURY CONCENTRATIONS

(µg Hg/g of Body Tissue)

DOSE ADMINISTERED mg Hg/Kg of B.Wt.	TISSUE CONCENTRATION $\bar{x}$	S.D.	n
0	0	0	6
2	1.57	0.59	14
4	3.33	0.44	8
5	5.66	1.60	6
6	5.95	1.21	8
8	7.79	1.25	6

APPENDIX Ic

MEAN BRAIN, EYE, MUSCLE MERCURY CONCENTRATIONS

(µg Hg/g tissue)

DOSE ADMINISTERED			TISSUE CONCENTRATION					
			BRAIN		MUSCLE		EYE	
t.u.	mgHg/Kg of B.Wt.	n	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	0	11	0	0	0	0	0	0
0.3	1.8	11	1.52	0.35	1.46	0.37	0.54	0.26
0.6	3.6	11	2.89	0.61	2.49	0.48	1.44	1.14
0.8	4.8	11	4.19	0.88	3.21	0.82	1.70	0.44